Dilute Solution Properties of Pectin

The colligative properties of various pectins in the fully protonated and neutralized forms were studied by membrane osmometry. Because van't Hoff plots passed through a minimum, apparently pectins behave as nonideal, dissociating macromolecules in solution. In approaching the $\lim_{c \to 0} \pi/c$, *where* c *is concentration, number-average molecular weights* ($\overline{M}_n$) *from osmometry approached the* $\overline{M}_n$ *determined from end-group titrations. Second virial coefficients of pectin aggregates in 0.05 M NaCl revealed their ratio of length to width was from 120 to 200. Counterion binding decreased with pectin dissociation and increasing percentage of ester groups in the neutralized form.*

PECTIN, predominantly a copolymer of α(1–4)-galacturonate and its methyl esters with about 20% neutral sugars, is important because it is a major structural polysaccharide in plants (*1*), a ubiquitous gel former and thickening agent in foods (2), and a nutritionally important food fiber (3). The inter- and intramolecular forces within the pectin structure are important in understanding how pectin functions in these systems. Thus, the colligative properties as ascertained by membrane osmometry (*O*) of various pectins were studied to better understand these forces.

Interestingly, pectic substances were among the first macromolecules studied by membrane osmometry (*4*). Previously, Owens et al. (*5*) found the number-average molecular weight, $\overline{M}_n$, in the range (1.8–3.9) $\times 10^4$ for protonated citrus pectins, whereas Pals and Hermans (*6*) found $\overline{M}_n$ to be 4.6×10^4 for the sodium salt of citrus pectin. More recently, Jordan and Brant (7) reported an $\overline{M}_n$ of 4.9×10^4 for protonated pectin. In all cases pectin appeared to follow the van't Hoff limiting law.

Measurements by gel chromatography (*8, 9*), light scattering (*7, 10*), and electron microscopy (*11*) indicated that pectins aggregate in solution. Aggregation is further supported by a preliminary observation that $\overline{M}_n$ values determined by end-group titration for pectins were significantly lower than those obtained by membrane osmometry (*12*). Recent advances in the design of high-speed membrane osmometers, with

increased sensitivity and reliability, together with a better theoretical understanding of aggregating systems (*13*), have prompted us to reexamine the osmotic properties of pectins to reconcile these various observations.

Theory

A plot of reduced osmotic pressure, π/c, against concentration, c, according to the van't Hoff limiting law (*14*) yields

$$\pi/c = RT(1/\overline{M}_n + Bc) \tag{1}$$

for a polydisperse system, $\overline{M}_n$ from the intercept of a straight line. (R is the gas constant and T is the absolute temperature.) For a polyelectrolyte, the second virial coefficient, B, obtained from the slope of a van't Hoff plot contains three contributions

$$B = B_I + B_{II} - B_{III} \tag{2}$$

B_I and B_{II} are positive contributions arising from the Donnan effect and polymer–polymer interactions, respectively, whereas B_{III} is a negative contribution due to preferential solvation effects (*13*).

So that structural information from equation 1 can be obtained, separation of the various contributions to B is necessary. Thus, protonated and neutralized pectins were measured in 0.05 M NaCl. Under these conditions, we assumed for the protonated form, which is a weak carboxylic acid

$$B_{I,H} \sim B_{III,H} \approx O \tag{3}$$

Thus, from equation 2

$$B_H \cong B_{II,H} \tag{4}$$

Here the subscript H refers to the protonated form of pectin.

Previously (*8*), we showed that the root mean square radii of gyration (R_g) of pectins measured over the pH range 3.7–7.3 are the same within experimental error. Thus

$$B_{II,Na} \approx B_{II,H} \tag{5}$$

Here, the subscript Na refers to the neutralized form. If we further assume

$$B_{III,Na} \approx O \tag{6}$$

Then

$$B_{I,Na} \cong B_{Na} - B_H \tag{7}$$

Given the above approximations, the axial ratio (L/d) of pectin can be obtained from solutions of protonated pectin by (*14*)

$$L/d = B_H\overline{M}_n/1000V_1V_2 \quad (8)$$

Here V_1 and V_2 are partial specific volumes of solvent and polymer, respectively, and L/d is the ratio of macromolecular length to diameter.

Furthermore, the effective charge (Z_{eff}) on neutralized pectins can be obtained from second virial measurements on protonated and neutralized pectins by (*14*)

$$Z_{eff} = [4(B_{Na} - B_H)\overline{M}_n^2 m_3/1000V_1]^{1/2} \quad (9)$$

Here m_3 is the molality of added salt, in this case NaCl.

Moreover, the fraction of dissociated counter ions, α, can be calculated from equations 10 and 11:

$$\alpha = Z_{eff}/Z_s \quad (10)$$

and

$$Z_s = \overline{DP}_n(1 - \epsilon)X_G \quad (11)$$

where Z_s is the stoichiometric charge on neutralized pectin, $\overline{DP}_n$ is the number-average degree of polymerization, ϵ is the mole fraction of galacturonate residues containing methyl esters, and X_G is the mole fraction of galacturonate residues in pectin.

Experimental Section

Materials. Commercial citrus pectins with degrees of methyl esterification (DM) 35, 58–60, and 70 were gifts from Bulmers Ltd., Hereford, England. Two other pectin samples, DM-37 and 73, were manufactured by Bulmers but were gifts from Drs. E. R. Morris and M. J. Gidley at Unilever. The DM-57 pectin was a gift from Sunkist Growers, Corona, CA. Poly(galacturonic acid) was from Sigma Chemical Co. Characterization and preparation of samples were reported previously (*8*) with minor modification. Samples to be neutralized with NaOH were dissolved in 0.01 M phosphate buffer (pH 6.1) containing 0.1 M ethylenediaminetetraacetic acid, titrated to pH 7 with 0.1 M NaOH, dialyzed against four changes of water for 48 h, centrifuged for 1 h at 30,000*g* to remove insoluble matter, and then lyophilized. Dialysis bags were Spectrapor with a molecular weight cutoff of 12,000.

Membrane Osmometry. Osmotic pressures were measured in a Knauer-type 1.00 membrane osmometer equipped with a thermostated cell (Utopia Instrument Co., Joliet, IL). The solvent was 0.05 M NaCl. Semipermeable membranes (Schleicher & Schuell, AC 62) were cellulose acetate with pore-size diameters between 50 and 100 Å. The osmometer cell was maintained at 35 ±

0.1 °C. The output from the electronic pressure transducer in the osmometic cell was monitored continuously by a potentiometric recorder. Recorder traces of π against time, which were flat and parallel to the base line, indicated no tendency for pectin to permeate the membrane. Flat and parallel traces of π were obtained 5–10 min after the third cell rinse with the polymer solution. Samples were measured in increasing order of concentration.

Initially pectins were dissolved at room temperature to a concentration of 1 g/dL in 0.05 M NaCl. The stock solutions were diluted serially and measured within 24 h of initial solvation. Measurements of π at $c < 0.1$ g/dL were scattered. Thus, experiments were conducted to obtain reliable values of π/c when $c \leqq 0.1$ g/dL.

On the assumption that a slow approach to equilibrium produced scattered π/c values for $c < 0.1$ g/dL, the approach to equilibrium was followed by measuring π for 0.6 g/dL pectin solutions as a function of time. The solvent was 0.047 M NaCl and 0.003 M sodium azide; samples were contained in capped bottles. These bottles were immersed in boiling water for 10 min at the start of the experiment and then kept in a water bath at 35 ± 1 °C prior to measuring π. The apparent number-average molecular weight, $\overline{M}_n^{app}$, was obtained from equation 1 with $B = 0$. On the final day of the experiments (days 8–15), the samples were diluted serially to obtain $\overline{M}_n$ and B.

Lastly, pectin solutions (1 g/dL) were heated for 10 min in boiling water as before and allowed to stand for 3 days at 35 °C. In the case of protonated pectin with a DM of 35 or 37, the data were too scattered to obtain $\overline{M}_n$ or B.

End-Group Titration. The $\overline{M}_n$ of pectins was determined also by the reaction of sodium chlorite with aldehyde end groups. This method was first developed for polysaccharides by Launer and Tomimatsu (*15*, *16*) and later applied specifically to pectins by Albersheim et al. (*17*). Galacturonic acid and rhamnose standards gave the same results within experimental error, provided the chlorite reaction was allowed to proceed for a minimum of 16 h. Within the precision of our measurements, $\overline{M}_n$ was the same for the protonated or neutralized pectins, although neutralized forms dissolved more readily. In several cases, the neutralized forms were heated to 100 °C for 10 min before allowing the chlorite reaction to proceed with refluxing. The results showed no significant change in $\overline{M}_n$.

Results and Discussion

Unheated Pectins. As indicated in Table I, $\overline{DP}_n$ by membrane osmometry for the unheated pectins was found to be significantly higher than values by end-group titration. The $\overline{M}_n$ values for the high-methoxy pectins (DM-70 and DM-72–73) in both acid and neutralized forms ranged between 3.5×10^4 and $4 - 10^4$. These values are comparable to those found by Owens et al. (*5*) but somewhat lower than the values obtained by Jordan and Brant (*7*) or Pals and Hermans (*6*). The van't Hoff plots are shown in Figures 1 and 2. Correlation coefficients from linear least squares were ≧0.97. If pectin aggregates as indicated by a value of $\overline{AGR}_n > 1$ ($\overline{AGR}_n$ is $\overline{M}_{n,0}:\overline{M}_{n,EGT}$, where EGT is end-group titration; *see* Table I), then at $c \leqq 0.1$ g/dL, π/c must decrease with increasing concentration until a minimum is reached. Initial studies at $c \leqq 0.1$ g/dL gave scattered values of π/c. Studies were initiated to see if scattered π/c values were caused by a slow approach to equilibrium.

Table I. $\overline{DP}_n$ and $\overline{AGR}_n$ for Unheated Pectins

DM	$\overline{DP}_n$			$\overline{AGR}_n$[b]	
	End-Group Analysis	*Osmometry*[a]			
		Na	*H*	*Na*	*H*
0	29.7± 4.0	39± 1	—	1.3±0.2	—
35	50.7± 9.0	183±11	—	3.6±0.9	—
37	32.8± 3.5	89± 1	—	2.7±0.3	—
57	65.9± 1.2	213± 1	219± 4	3.2±0.6	3.3±0.1
58–60	66.0± 8.6	252±34	201±10	3.8±1.1	3.0±0.5
70	67.6±10.9	218±10	200± 4	3.2±0.7	3.0±0.5
72–73	60.0± 1.2	219± 6	213± 3	3.7±0.2	3.6±0.1

[a] The values were determined within 24 h of solution preparation.
[b] $\overline{AGR}_n = \overline{M}_{n,0}:\overline{M}_{n,EGT}$ (EGT is end-group titration).

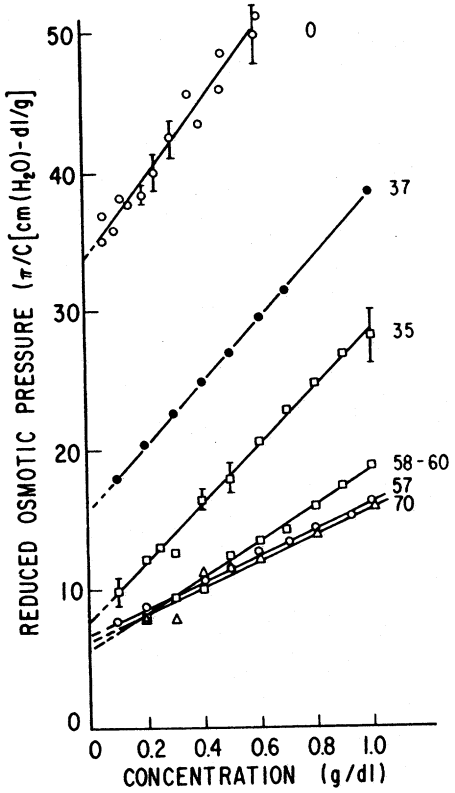

Figure 1. van't Hoff plots for sodium salts of unheated pectins. Key: ⊖, DM = 0; ●, DM = 37; ⊟, DM = 35; □, DM = 58-60; ○, DM = 57; △, DM = 70.

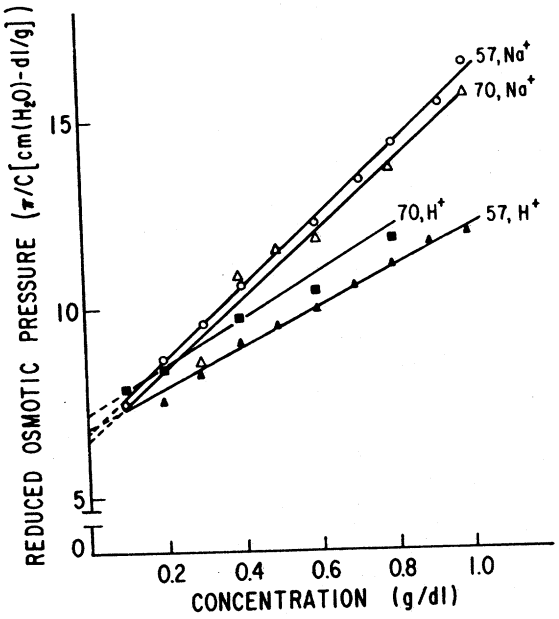

Figure 2. van't Hoff plots for protonated and sodium salts of unheated pectins. Key: ○, *DM = 57, Na^+;* △, *DM = 70, Na^+;* ■, *DM = 70, H^+; and* ▲, *DM = 57, H^+.*

Dissociation of Heated Pectins. With one exception, $\overline{M}_n^{app}$ for c = 0.6 g/dL, as calculated from equation 1 with $B = O$, decreased slowly with time. We concluded that such was probable by fitting $\overline{M}_n^{app}$ against time by the linear least-squares procedure. Correlation coefficients (Table II) were moderately to highly negative, except for the low-methoxy protonated pectins. Examination of the data in Figure 3 reveals that the high- (DM-70 and 72–73) and medium- (DM-57 and 58–60) methoxy pectins in the protonated form and the low-methoxy pectins (DM-35 and 37) in the sodium form required several days of incubation before a decrease in $\overline{M}_n^{app}$ was observed. Thus, for these cases, data were not fitted to a linear least-squares equation in view of the lag time between incubation and an observed decrease in $\overline{M}_n^{app}$. In the case of low- and medium-methoxy pectins in the sodium form, $\overline{M}_n^{app}$ appeared to decrease linearly with time, whereas the low-methoxy pectin in the protonated form remained unchanged. These trends are shown more clearly in Figure 4, in which the data were normalized for molecular weight by the ratio ($\overline{M}_{n,t}^{app}$:$\overline{M}_{n,0}^{app}$) (i.e., $\overline{M}_n^{app}$ at time t divided by the apparent value extrapolated to $t = 0$). The overall rate ($d\overline{M}_n^{app}/dt$) in order of DM is medium > high > low. Furthermore, at constant DM, the rate is more rapid for the sodium salt than the protonated form.

Table II. Decrease of $\overline{M}_n^{app}$ as a Function of Time (Days) in 0.047 M NaCl and 0.003 M NaN_2 for Pectins

	H			*Na*		
DM	$-k^a \times 10^{-2}$	$b^b \times 10^{-3}$	*Corr Coeff*	$-k \times 10^{-2}$	$b \times 10^{-3}$	*Corr Coeff*
35	−1.7	30.4±0.4	+0.68	0.5	16.3	−0.998
37	0.5±1.0	27.0±0.7	−0.31	0.9±0.4	15.3±0.3	−0.840
57	2.7±0.3	32.5±0.2	−0.98	2.5±0.3	27.6±0.3	−0.980
58-60	3.8±0.7	31.4±0.4	−0.97	—	—	—
70	3.0±0.4	39.1±0.6	−0.89	1.8±0.5	26.7±0.4	−0.980
72-73	1.7±1.0	35.7±0.5	−0.79	1.4±0.2	25.5±0.1	−0.998

[a] *k* is the slope.
[b] *b* is the intercept.

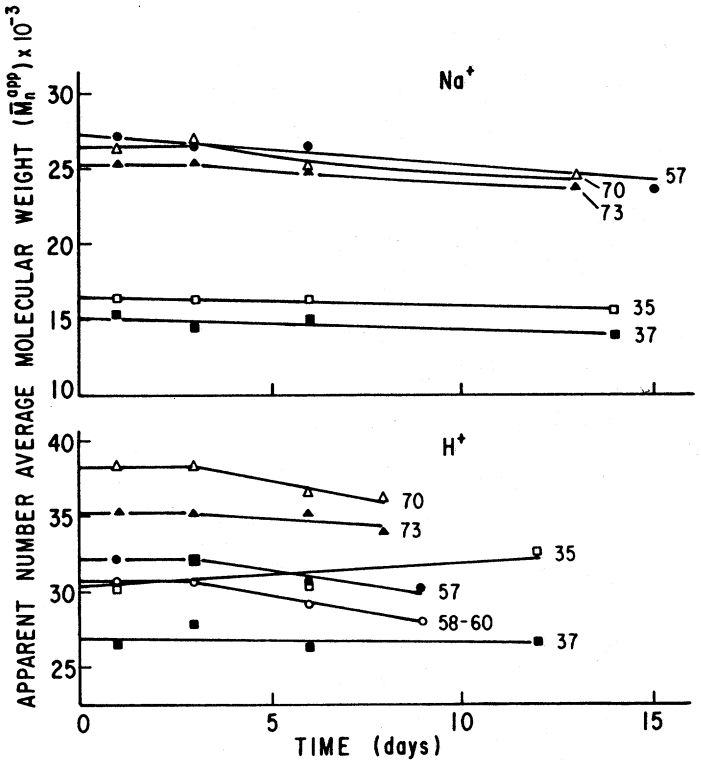

Figure 3. $\overline{M}_n^{app}$ as a function of time for protonated and sodium salts of pectin. The concentration was 0.6 g/dL. Top (for Na^+): ●, DM = 57; △, DM = 70; ▲, DM = 73; □, DM = 35; and ■, DM = 37. Bottom (for H^+): △, DM = 70; ▲, DM = 73; □, DM = 35; ●, DM = 57; ○, DM = 58-60; and ■, DM = 37.

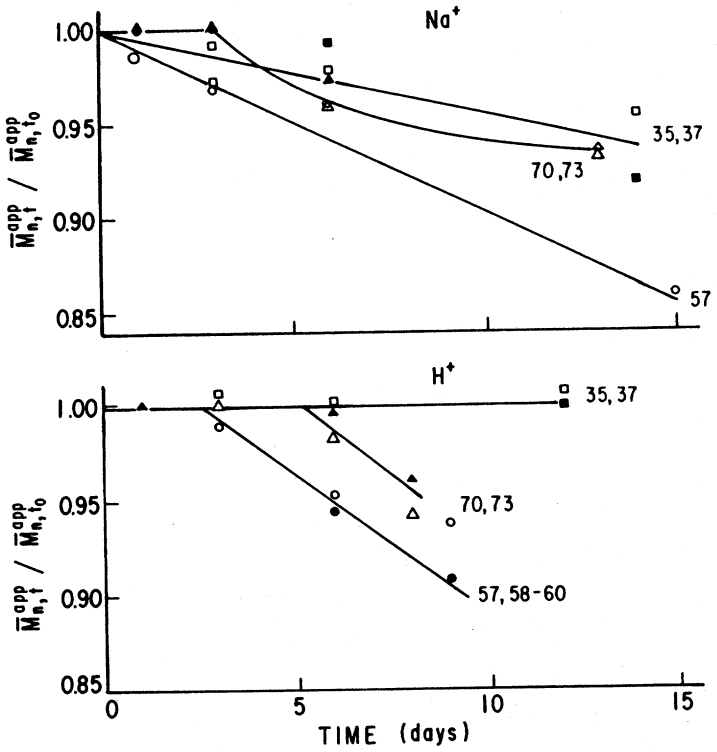

Figure 4. $\overline{M}_{n,0}^{app}:\overline{M}_{n,t}^{app}$ *as a function of time for protonated and sodium salts of pectin. The concentration was 0.6 g/dL. Top (for* Na^+*):* □ *and* ■*, DM = 35 and 37;* △ *and* ▲*, DM = 70 and 73; and* ○ *and* ●*, DM = 57. Bottom (for* H^+*):* □ *and* ■*, DM = 35 and 37;* △ *and* ▲*, DM = 70 and 73; and* ○ *and* ●*, DM = 57 and 58–60.*

Comparison of $\overline{DP}_n$ for Unheated and Heated Pectins. A decrease in $\overline{M}_n^{app}$ could be caused by changes in molecular weight, second virial coefficient, or a combination of the two. Thus, after following the decrease in $\overline{M}_n^{app}$ for 8–15 days, samples were diluted on the final day and van't Hoff plots obtained (Figure 5). In one case, the sodium salt with DM-58–60, the van't Hoff plot was obtained after treating the sample according to the procedure under End-Group Titration. A comparison of the data in Table III with that in Table I revealed that $\overline{AGR}_n$ for the heated and equilibrated pectins was lower than for the unheated pectins. A comparison of second virial coefficients (*B* in Table IV) revealed these coefficients were greater for the heated pectins. Generally, for comparable samples and conditions, the percentage change in $\overline{M}_n^{app}$ is less than the percentage change in $\overline{M}_n$. For example, $\overline{M}_n^{app}$ for the medium-methoxy, protonated pectins dropped by about 8% (Figure

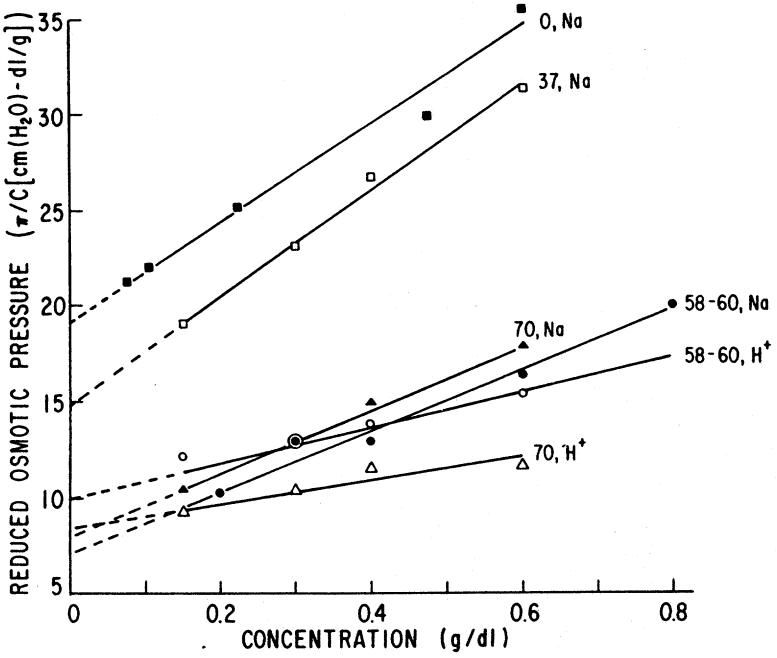

Figure 5. Typical van't Hoff plots for heated and equilibrated pectins after 8–15 days. Key: ■, DM = 0, Na^+; □, DM = 37, Na^+; ▲, DM = 70, Na^+; ●, DM = 58–60, Na^+; ○, DM = 58–60, H^+; and △, DM = 70, H^+.

Table III. $\overline{DP}_n$ and $\overline{AGR}_n$ for Heated Pectins

	$\overline{DP}_n$				
		Osmometry		$\overline{AGR}_n$[a]	
DM	End-Group Analysis	Na	H	Na	H
0	29.7± 4.0	51± 1	—	1.7±0.3	—
35	50.7± 9.0	126± 1	—	2.5±0.5	—
37	32.8± 3.5	94± 3	—	2.9±0.4	—
57	65.9± 1.2	186± 2	155± 3	2.8±0.5	2.4±0.5
58–60	66.0± 8.6	196±13	146± 4	3.0±0.6	2.2±0.3
70	67.6±10.9	178± 7	170± 7	2.6±0.5	2.6±0.5
72–73	60.0± 1.2	206±11	171±10	3.4±0.2	2.9±0.2

[a] $\overline{AGR}_n = \overline{M}_{n,0}/\overline{M}_{n,EGT}$.

4) in 8 days, whereas $\overline{M}_n$ decreased by about 30% (cf. Tables I and III). Interestingly, for comparable samples, the percentage drop in $\overline{M}_n$ of the protonated form is either equal to or greater than the percentage change in $\overline{M}_n$ for the sodium form. For high- and medium-methoxy pectins, a comparison of sodium and protonated forms also revealed no significant difference in $\overline{AGR}_n$ for the unheated pectins (Table I).

Comparison of Axial Ratios (L/d) and Counterion Binding for Heated and Unheated Pectins. When L/d was calculated by using equation 8, a value of 0.62 was taken for V_2 of the pectins and 0.56 for poly(galacturonic acid) (*18*). V_1 was taken as that of water. For the medium-methoxy protonated pectins, L/d appears to have increased somewhat with heating and equilibration, whereas heating and equilibration appeared to have no effect on the axial ratio of high-methoxy pectin (Table IV).

The low-methoxy protonated pectin gave scattered data when van't Hoff plots were attempted. The solubility of poly(galacturonic acid) was too limited to obtain van't Hoff plots. Nevertheless, L/d and B_H were calculated by the following procedure. PG was assumed to be a rod (*19*) with a virtual bond length of 5 Å (i.e., the length of a monomer unit along the x axis) (*20*). Thus, the overall length ($\underline{L}$) was calculated from the product of the virtual bond length and the $\overline{DP}_n$. Furthermore, when the diameter (d) was estimated to be 10 Å, an approximate value of L/d could be obtained and an approximate B_H calculated from equation 8 (Table IV).

Unlike the protonated pectins, van't Hoff plots were obtained for all the neutralized pectins, so that values of $1 - \alpha$ (fraction of bound counterions) were obtained from equations 9–11. A value of 0.78 was used for X_C in equation 11 (*8*). Counterion binding decreased for the heated and equilibrated pectins compared to the unheated (Table IV). Furthermore, for either heated or unheated pectins, $1 - \alpha$ increased with decreasing DM due to decreasing charge density. Decreasing the fraction of esterified carboxyl groups (ϵ) is analogous to the progressive neutralization of a poly(acrylic acid) (*21*) (Figure 6) or increasing the percentage of charged residues in a copolymer containing a mixture of charged and uncharged comonomers (*22*).

Concentration-Dependent Dissociation. In light of the findings that pectin dissociation required activation and that, in four of six cases, several days of lag time ensued between activation and an observed decrease in $\overline{M}_n^{app}$, a series of critical experiments were performed to test the hypothesis that pectins undergo a concentration-dependent dissociation. The experiments are described under End-Group Titration. Remarkably, the minima in the van't Hoff plots of Figure 7 are evidence that neutralized pectins with a DM between 73 and 35 and protonated

Table IV. Structural Information from Second Virial Coefficient (B)

	Unheated				*Heated*			
	H		*Na*		*H*		*Na*	
DM	B × 10^4 (mL/g^2-mol)	L/d ($\times 10^{-2}$)	B × 10^4 (mL/g^2-mol)	$1-\alpha$	B × 10^4 (mL/g^2-mol)	L/d ($\times 10^{-2}$)	B × 10^4 (mL/g^2-mol)	$1-\alpha$
0	14[a]	0.2[a]	106±6	0.74	11[a]	0.3[a]	99±7	0.65
35	—	—	80±1	—	—	—	106±1	—
37	—	—	86±4	—	—	—	105±5	—
57	20±1	1.2	36±1	0.69	31±2	1.7	69±1	0.53
58–60	26±5	1.5	49±4	0.61	35±3	2.0	61±7	0.56
70	20±1	1.2	35±4	0.50	23±4	1.2	63±6	0.32
72–73	26±1	1.6	47±3	0.44	29±7	1.8	73±6	0.17

[a]The value was calculated by assuming a rod with residue virtual bond length of 5 Å and a diameter of 10 Å.

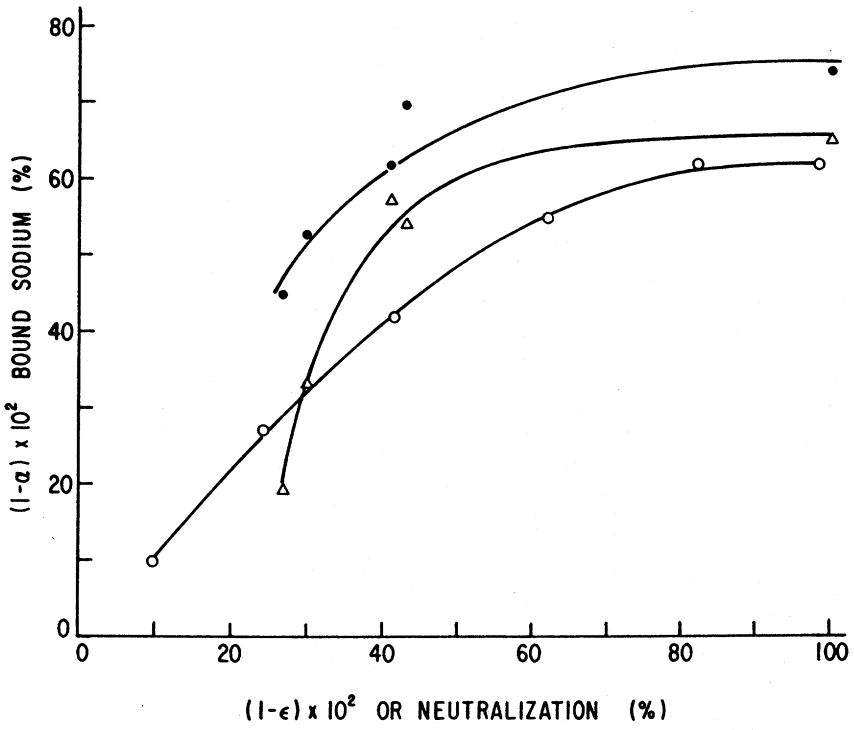

Figure 6. Counterion binding as a function of degree of esterification [(1 − ε) × 10²] for heated and unheated pectin. Data for 0.015 N poly(acrylic acid) were taken from reference 21. Key: ●, unheated pectin; △, heated pectin; and ○, 0.0151 N poly(acrylic acid).

pectin with DM-72–73 undergo concentration-dependent dissociation in the concentration range below 0.1 g/dL. Although not shown, the DM-70, 57, and 58–60 pectins behave similarly. Values of π/c at the intercept were calculated from $\bar{M}_n$ values obtained by end-group titration.

We note that in the absence of activation and the observance of a lag time, our results were consistent with the osmotic pressure results of previous investigators (5–7). Only after activation and observance of a lag time were we able to obtain π/c values sufficiently free of scatter in the <0.1-g/dL range to observe reproducible minima in van't Hoff plots. Thus, we were able to recognize the concentration-dependent dissociation of pectin, whereas others failed.

In cases where dissociation is accompanied by large free-energy changes, the concentration range of nonlinearity should be rather narrow. Thus, the van't Hoff plots in Figure 7 could arise from dissociating

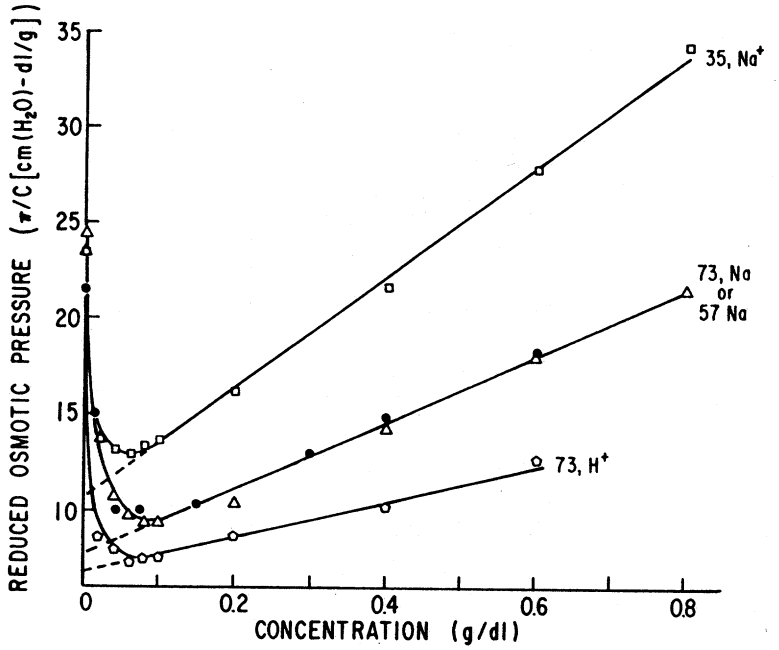

Figure 7. van't Hoff plots demonstrating concentration-dependent dissociation of pectin. Values at the intercept were calculated from end-group titrations. Key: □, DM = 35, Na^+; ● and △, DM = 73 or 57, Na^+; and ⬠, DM = 73, H^+.

systems that form nonideal aggregates (*13*) under the influence of relatively large free-energy changes.

Acknowledgment

Reference to brand or firm names does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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